THE FUNCTION OF THE CONTRACTILE VACUOLE IN *PARAMECIUM CAUDATUM;* WITH SPECIAL REFERENCE TO THE EXCRETION OF NITROGENOUS COMPOUNDS.[1]

J. H. WEATHERBY,

Zoölogical Laboratory of the Johns Hopkins University.

Introduction.

Ehrenberg (1838) was probably the first to consider the function of the contractile vacuole. He asserted that it is a spermatic gland; but no evidence has been found in support of this view.

Lieberkühn (1836), Claparède (1834), Lachmann (1856), Siebold and Stannius (1854), and Pritchard (1861) held that it is a rudimentary heart, which, by its pulsations, produces the circulation of a body-fluid throughout the organism. The demonstration by Jennings (1904) that the vacuole communicates directly with the exterior and discharges its contents into the surrounding medium definitely eliminates such an explanation.

Haeckel (see Kent, 1880, p. 69), Maupas (1863), Bütschli (1887–89), Ehrmann (1894), and others contend that it is a respiratory organelle, or a mechanism for the removal of some of the end products of oxidation; but insufficient evidence has been found to warrant the acceptance of this view.

Stein and Schmidt (see Kent, 1880, p. 69), Griffiths (1888), Calkins (1909), Khainsky (1910), Woodruff (1911), Minchin (1912), Howland (1924), Nowikoff (1908), Shumway (1917), Riddle and Torrey (1923), Flather (1919), and Marshall (1921) believed the vacuole to be an excretory organelle. This view

[1] This paper is a portion of an essay submitted to the Board of University Studies of the Johns Hopkins University in partial fulfillment of the requirements for the degree of Master of Arts. The writer wishes to acknowledge his indebtedness to Professor S. O. Mast, who suggested the problem, and under whose personal supervision the work was done; and to Professor E. K. Marshall of the Department of Physiology of the Johns Hopkins Medical School who offered many helpful suggestions in connection with the literature on the enzyme, urease, and the xanthydrol test for urea.

is widely accepted, and is supported to some extent by experimental evidence. In certain instances, the term excretion is limited to mean only the expulsion of a fluid from the cell, as in Carter's (1861) observations. Generally, however, this theory assigns to the vacuole the function of a renal organ of some kind.

Hartog (1888), Calkins (1901), Zülzer (1910), Doflein (1911), and others maintain that the vacuole is an organelle for regulating the hydrostatic pressure within the cell, or a mechanism for removing the excess water which is taken into the body in feeding and through the cell membrane by osmosis. Stempell (1914) constructed a mechanical system which shows clearly that osmosis can be made the causal agent for producing intermittent discharge of a fluid from such a system, but this system was doubtless not intended to be compared with the vacuole in any respect other than the pulsating effect.

The literature concerned with the function of the contractile vacuole consequently reveals no conclusive evidence in support of one theory to the exclusion of all others. The consensus of the evidence, however, seems to indicate that the vacuole is an organelle concerned either with the removal of waste products of metabolism, or with the removal of excess water which accumulates in the organism as a result of endosmosis and feeding. The experiments described in the following pages have a direct bearing on the question as to which of these obtains. These experiments consist of attempts to ascertain the nature of the nitrogenous excretion products of metabolism in *Paramecium caudatum*, and whether or not they are excreted through the contractile vacuole.

The Nature of the Nitrogenous End Products in *Paramecium*.

The nitrogenous end products of metabolism in organisms vary in their nature according to the type of organism. For example, in man the bulk of the nitrogen is eliminated as urea, considerably less as uric acid and amino acids, and a very small part as free ammonia; while in the birds and reptiles the bulk of the nitrogen is eliminated as uric acid.

In connection with protozoa Griffiths (1888) made the statement, based on his own experiments, that the vacuole performs the function of a kidney, and that its secretions are "capable of yielding microscopic crystals of uric acid." As material for these experiments he used Amœba, Paramecium, and Vorticella, in mass cultures. In some of the experiments a number of amœbæ were placed on a slide and subjected to the murexide test. The development of reddish-purple color indicated the presence of uric acid. In describing these experiments Griffiths says (p. 132): "After the addition of alcohol minute flakes could be distinctly seen floating in the fluid of certain vacuoles. Bearing in mind the murexide reaction, there is every reason to believe that these flakes are nothing more or less than minute crystals of uric acid." These experiments were repeated many times, generally with positive results, indicating the presence of uric acid. At times, however, the vacuole was found not to contain the slightest trace of uric acid.

Howland (1924) repeated these experiments using several specimens of *Centropryxis* and *Amœba verrucosa*. Cells observed in a dark field immediately after the addition of alcohol did not show the crystals of uric acid in the distended vacuoles, nor did cells after the addition of ammonia show the characteristic murexide reactions, either in the vacuoles or in the culture medium immediately surrounding the organisms. These experiments were conducted during a period of five weeks, always with negative results. Paramecia were subjected to the same test, also with negative results. Howland made use of the Benedict blood-filtrate test for uric acid on cultures of paramecia and amœbæ with positive results. The depth of the color developed varied with the age of the cultures, the older ones giving a deeper color. This indicates that uric acid was eliminated in some way by the organisms.

The question then arises: Is nitrogen excreted by protozoa as ammonia, urea, uric acid, or a combination of these substances? In an effort to answer this question the following experiment was conducted. A large number of paramecia were thoroughly washed, placed in spring water that was free from ammonia and urea, left for a time, and removed by filtration. The

filtrate was then tested for ammonia and urea as described below. The possible presence of uric acid was not investigated at this time.

The paramecia were washed as follows: Culture fluid containing the organisms was poured into a long-necked bottle of approximately one liter capacity until it was filled to within about 5 cm. of the top. Then spring-water was added carefully so as to avoid mixing until the bottle was entirely full. Within five or ten minutes under these conditions the paramecia usually aggregated in very great numbers near the surface of the spring-water. When they had thus collected at the top of the bottle, the surface water containing them was removed with a pipette, and more spring-water added. This process was repeated until most of the animals had been taken from the culture fluid, usually three or four times. The bottle was then emptied, and the water containing the paramecia put into it, after which it was filled with spring-water. The organisms were removed as before. The paramecia were thus washed in fresh spring-water three or four times, after which they were usually found to be free from all heavy debris and large bacterial masses. Smaller organisms which were removed from the bottle with the paramecia were separated from the paramecia by further washing on filter paper. The paper used was about 20 cm. in diameter, and was selected so that the pores were small enough to retain the paramecia, but large enough to allow the smaller organisms to pass through. A liter of water, or more if necessary, was used for this part of the washing process.

After the paramecia had been thus washed they were put into a clean glass beaker; then spring-water was added until the number per cubic centimeter was reduced to from 500 to 2,500 individuals. This was ascertained by counting the numbers in several one cubic centimeter portions and averaging the results obtained. The paramecia were allowed to remain in this water for periods of time ranging from eighteen to thirty-six hours, after which they were removed by filtration.

A portion of the filtrate was tested for ammonia by Nesslerization and the rest for urea by methods described below.

In twenty-two of the twenty-five experiments positive tests

were obtained for ammonia by Nesslerization. In the three in which no ammonia was found the paramecia had been in the water for a period of thirty hours or less. In other experiments in which the length of time was thirty-six hours or more, ammonia was invariably found. This indicates that either ammonia was eliminated in such small amounts that more than thirty hours were required for the concentration to rise sufficiently high to be detected by Nesslerization; or that no ammonia was eliminated as such, the positive test being due to that formed from the hydrolysis of some other excretion product. The latter seems the more probable, for, if ammonia was excreted, the length of time necessary for its concentration to rise sufficiently high to be detected should bear an inverse relation to the number of paramecia per unit volume of water. No relation of this kind was found to exist. The ammonia appeared after thirty to thirty-six hours in all the experiments regardless of the number of animals present. The maximum variation, then, in the length of time necessary for ammonia to make its appearance was twenty per cent., while the variation in the number of paramecia in these same experiments was one hundred per cent. or more. The absence of ammonia in three experiments, and its presence in all the others can be explained if it is assumed that there were too few bacteria present in the three to produce hydrolysis of the more complex excretion products, while in the other experiments there were enough bacteria present. That ammonia can be produced in this way was demonstrated by inoculating a dilute solution of urea with culture fluid. After the solution had been allowed to stand for several hours it gave a positive test for ammonia with Nessler's reagent, indicating that hydrolysis had taken place.

Many tests were made of the materials used in these experiments to prevent the possible introduction of errors. The spring-water was tested for ammonia. The sensitivity of Nessler's reagent was ascertained by finding the greatest dilution possible at which a definite indication of ammonia could be obtained. This dilution was found to be approximately one part in two million. The filter paper on which the paramecia were washed was tested for ammonia.

To ascertain whether or not ammonia is present in the fluid of the vacuole, fifteen experiments were conducted in which Nessler's reagent was injected into the organism. The apparatus used in making these injections consisted of the micropipette developed by Taylor (1925) mounted on the micromanipulator developed by Chambers (1922). The process of injection was performed with the paramecium held by surface tension in a hanging drop of water. The cover-glass bearing the organism formed the top of a cell, the front of which was left open to allow the micropipette entrance. The tip of the pipette was bent up at a right angle to the main shaft to facilitate the injection of the organism suspended on the lower surface of the cover-glass.

In twelve of the injections the contents of the pipette were discharged into the vacuole. In three the pipette did not penetrate the vacuole, but discharged its contents into the cytoplasm in the immediate vicinity of the vacuole. In every test the reagent, which is highly caustic, caused the immediate solution of the whole organism with the exception of the nucleus, which remained intact for a short time before it too was dissolved. In the three tests in which the pipette did not penetrate the vacuole, the surrounding cytoplasm was dissolved as before, but the membrane around the vacuole remained intact for a short time. After several seconds the membrane was dissolved, causing the contents of the vacuole to be emptied into the solution of Nessler's reagent in which it was floating. In none of these tests was the characteristic straw color observed which, in the presence of Nessler's reagent, indicates ammonia. It seems, then, that if ammonia is present in any part of the organism its concentration is below the sensitivity of the reagent. All of these experiments seem to indicate then that very little if any of the nitrogen found in the excretion products of *Paramecium* is excreted in the form of ammonia.

The test for urea referred to above was made as follows: Urease, a specific enzyme for urea, hydrolyzing it into ammonia and carbon dioxide, was added to the portion of the filtrate not used for the test for ammonia in each of the twenty-five experiments mentioned. They were then left for several hours, after

which they were tested for ammonia by Nesslerization. Ammonia was found in the filtrate from every experiment, and generally in higher concentrations than it was in the portions to which no urease had been added. This increased ammonia content after hydrolysis may, then, with a reasonable degree of certainty, be interpreted as indicating that urea from some source had been hydrolyzed with the subsequent production of ammonia. The fact that the three filtrates which gave no indication of ammonia before hydrolysis, gave a distinctly positive test for ammonia after hydrolysis, is alone conclusive in so far as the action of urease is known to be limited to the hydrolysis of urea. Since paramecia were the only organisms present in the water in any considerable numbers, the source of this urea must be attributed to them. It therefore seems evident that in *Paramecium* at least some of the nitrogen is excreted in the form of urea.

Is Urea Eliminated by the Contractile Vacuole?

In an effort to answer this question the xanthydrol precipitation test for urea, described by Fosse (1913) and modified to suit conditions of this experiment, was made by injecting the reagent into the vacuoles. The modified reagent consisted of three to five drops of a ten per cent. solution of xanthydrol in methyl alcohol, in 1 cc. glacial acetic acid. This reagent, in the presence of urea, precipitates long, needle-like crystals of di-xanthyl urea which may be easily recognized. The sensitivity of the modified reagent was found by injecting it, with the aid of the apparatus described above, into a drop of a solution of urea of known concentration. The solution of urea used was successively diluted until the urea content was so low that no precipitate could be observed. It was found that one or more parts of urea in twelve thousand could be detected. The process of injecting the reagent into the drop of solution was observed under a microscope.

Considerable annoyance was encountered in attempting to inject the contractile vacuole of *Paramecium* with the xanthydrol reagent in that fumes from the acetic acid in the pipette killed the organism before the injection could be made. This difficulty

was finally overcome by drawing into the pipette a very small amount of paraffine oil after the pipette had been filled with the reagent. The oil is chemically inert under ordinary conditions and served the purpose very well.

The effect of the reagent on the five paramecia successfully injected was quite striking. The animal was fixed immediately. It assumed an almost hyaline appearance with the exception of the nucleus, food granules, and numerous short, thick crystals which are normally found throughout the body. The contractile vacuole disappeared completely. No trace of the characteristic needle-like crystals of di-xanthyl urea, which are precipitated by xanthydrol in the presence of urea, were found either in that part of the organism in which the vacuole is usually situated, or in the liquid surrounding the organism. Some of these observations were made under an apochromatic oil immersion lens system. It seems from this, then, that if urea is present in the fluid of the vacuole its concentration is too low to be detected with the reagent used, that is, one part in 12,000.

Now the question arises as to whether or not all of the urea excreted could be eliminated by the contractile vacuole if the concentration is as low as this. If not, then it is evident that the contractile vacuole does not function specifically in the excretion of nitrogen, and if this is true it is not an excretory organelle in the ordinary sense of the term.

The concentration of urea that should be in the fluid of the vacuole, if all of it is eliminated through it, was ascertained in the following manner. Maupas (1883) found that the vacuoles of *Paramecium aurelia* evacuate a quantity of water equal to the volume of the entire organism in forty-six minutes at twenty-seven degrees. It was assumed in making these calculations that the relative quantity of water evacuated by *Paramecium caudatum* is approximately equal to that evacuated by *Paramecium aurelia* during the same period and at the same temperature. The average volume of *Paramecium caudatum* was assumed to be that of a cylinder 150 microns long and 35 microns in diameter, and the diameter of the vacuole when distended 10 microns.

On the basis of these assumptions and the observations of

Maupas, the volume of water evacuated by a definite number of paramecia in a definite period of time was calculated. From this the concentration of urea that would be in the fluid of the vacuole, if its function is excretory, was computed. The results of these calculations show that the concentration of urea in the fluid of the vacuole would have to be of the order of one part in two or three thousand to eliminate through it the calculated amount of urea.

The reagent injected into the vacuole is, as previously stated, sensitive to one part of urea in twelve thousand. Since there was in these injections no indication of the presence of urea, it is evident that the results of these experiments are in opposition to the theory that the vacuole is an organelle whose function is the removal of the nitrogenous waste products of metabolism, unless it functions specifically in eliminating uric acid, which is not probable. If, then, it is true that the contractile vacuole functions either as an excretory organelle or a mechanism for regulating the hydrostatic pressure within the cell, it is evident that the results support the latter.

Summary

1. The presence of ammonia and urea in *Paramecium* cultures has been demonstrated.

2. Ammonia is due to the hydrolysis of urea, and is not excreted as such.

3. Nitrogen is eliminated in the form of urea.

4. All the urea excreted can not be eliminated through the vacuole.

5. The function of the vacuole is not the elimination of nitrogenous waste products of metabolism, but is probably the regulation of the hydrostatic pressure within the cell.

BIBLIOGRAPHY.

Bütschli, O.

1887–89 Die contractilen Vacuolen. Bronn's Thier-reich, Protozoa, Bd. 1, S. 1411–1459.

Calkins, G. N.

1901 The Protozoa. New York. 347 pp.

1909 Protozoölogy. New York. 349 pp.

Carter, H. J.

1861 Notes and Corrections on the Organization of Infusoria. Annals and Magazine of Natural History, 3d series, Vol. 8, pp. 281–290.

Claparède, E.

1854 Ueber Actinophryns eichhornii. Müller's Archiv für Anatomie, Physiologie und Wissenschaftliche Medizine.

Chambers, R.

1922 New Apparatus for the Dissection and Injection of Living Cells. Anat. Rec., Vol. 24, pp. 1–19.

Doflein, F.

1911 Lehrbuch der Protozoenkunde.

Ehrenberg, C. G.

1838 Die Infusions Thierchen als vollkommene Organismen. Leipzig: Voss., Bd. 18, S. 547.

Ehrmann, P.

1894 Ueber die kontractilen Vacuolen der Infusorien. Sitzungsberichte der naturforschende Gesellschaft zu Leipzig. Bd. 21, S. 89–102.

Flather, M. D.

1919 The Influence of the Glandular Extracts upon the Contractile Vacuoles of *Paramecium caudatum*. Biological Bulletin, Vol. 37, pp. 22–39.

Fosse, R.

1913 Sur l'identification de l'urèe et sa précipitation de solution extrêment diluèes. Comptes Rendus, t. 137, pp. 948–957.

Griffiths, A. B.

1888 A Method of Demonstrating the Presence of Uric Acid in the Contractile Vacuoles of Some of the Lower Organisms. Proceedings of the Roya Society of Edinburgh, V. 16, pp. 131–135.

Haeckel, E. H.

See Kent, 1880. A Manual of the Infusoria. London. 472 pp.

Hartog, M. M.

1888 Preliminary Notes on the Functions and Homologies of the Contractile Vacuole in Plants and Animals. Report of the British Association for the Advancement of Science. Bath. pp. 714–716.

Howland, Ruth B.

1924 On Excretion of Nitrogenous Waste as a Function of the Contractile Vacuole. Journal of Experimental Zoölogy, Vol. 40, pp. 231–250.

Jennings, H. S.

1904 A Method of Demonstrating the External Discharge of the Contractile Vacuole. Zoölogische Anzeiger, Bd. 27, S. 656–658.

Khainsky, A.

1910 Zur Morphologie und Physiologie einiger Infusorien (*Paramecium caudatum*) auf Grund einer neuen histologischen Methode. Archiv für Protistenkunde, Bd. 21, S. 1–60.

Lachmann, K. F.

Ueber einiger neu entdekte Infusorien und über contractilen Blasen bei den Infusorien. Verhandlung des Naturhistorische Vereins der preussen Rheinlande, Bd. 16, S. 66–68; 91–93.

Lieberkühn, N.

1856 Beiträge zur Anatomie der Infusorien. Archiv für Anatomie und Physiologie.

Marshall, Chas. E.

1921 Microbiology.

Maupas, E.

1883 Contribution a l'etude morphologique et anatomique des infusoires ciliés. Archives de Zoologie Expérimental et Générale, 2 serie, t. 1, pp. 427–664.

Minchin, E. A.

1912 An Introduction to the Study of the Protozoa.

Nowikoff, M.

1908 Ueber die Wirkung des schildrusen Extrahts und einiger anderer Organstoffe auf Ciliaten. Archiv für Protistenkunde, Bd. 11–12, S. 309–325.

Pritchard, A.

1861 A History of the Infusoria. London. 986 pp. (The contractile vesicle, pp. 312–326.)

Riddle, M. C., and Torrey, H. B.

1923 The Physiological Response of *Paramecium* to Thyroxin. Anatomical Record, Vol. 24, p. 396.

Shumway, U.

1917 The Effect of a Thyroid Diet on *Paramecium*. Jour. Exp. Zoöl., Vol. 22, pp. 529–562.

Siebold, C. Th., and Stannius, H.

1854 Comparative Anatomy. Translated and edited by Waldo I. Burnett.

Stempell, W.

1914 Ueber die Funktion der pulsierende Vacuole und einen Apparat zur Demonstrationen derselben. Zoölogischer Jahrbücher, Abteilung für Allgemeine Zoölogie und Physiologie der Tiere. Bd. 34, S. 437–478.

Taylor, C. V.

1925 Improved Micromanipulation Apparatus. University of California. Publications in Zoölogy, Vol. 26, pp. 443–454.

Woodruff, L. L.

1911 The Effect of Excretion Products of *Paramecium* on its Rate of Reproduction. Jour. Exp. Zoöl., Vol. 10, pp. 557–581.

Zülzer, Margaret.

1910 Ueber den influss des Meerwassers auf die pulsierende Vacuole. Archiv für Entwicklungs-mechanik der Organismen, Bd. 29, S. 632–640.